Engineering slow acting mono-Zn variants of metallo β-lactamases as a crystallographic and spectroscopic platforms for drug discovery

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Antibiotic resistance is a persistent threat in the clinic. The widespread use of β-lactam containing antibiotics has put evolutionary pressure on bacteria to develop an extensive library of enzymes that degrade these compounds. This family of enzymes, known as β-lactamases, are pervasive, adaptive, and omnipresent. Of particular concern are the metallo-β-lactamases such as New Dehli Metallo-β-lactamase (NDM), and VIM-2, as they readily degrade many of our last-resort antibiotics. One strategy for overcoming the threat of β-lactamases is the development of β-lactamase inhibitors and co-administering them with β-lactam containing antibiotics, as inhibiting the β-lactamase should preserve the integrity of the antibiotic. Unfortunately, there are currently no FDA approved inhibitors for the NDM or VIM families of β-lactamases. While several members of these families of proteins have been successfully crystalized and structures have been deposited into the PDB, all of the structures to date have only successfully captured β-lactam containing compounds in a hydrolyzed state, as the rate of hydrolysis is much faster than the timescale required for crystallization. The Page lab has engineered slow acting mono-Zinc variants of NDM-4 and VIM-20 for use as a crystallographic and spectroscopic platform to probe the pre-hydrolyzed enzyme-substrate complex. A crystal structure of our mono-Zinc version of NDM-4, called NDM-X has been solved at 1.4Å resolution with minimal structural changes relative to native NDM-4. A 10,000X reduction in activity shows promise for future crystal soaking experiments.